

Intronic SNPs of *TP53* gene in chronic myeloid leukemia: Impact on drug response

K. Sailaja, V. R. Rao^{1,2},
Satish Yadav¹,
R. Rajasekhar Reddy¹,
D. Surekha,
D. Nageswara Rao,
D. Raghunadharao³,
S. Vishnupriya

Department of Genetics, Osmania University, ¹Anthropological Survey of India, Mysore, ²Department of Anthropology, University of Delhi, Delhi, ³Nizam's Institute of Medical Sciences, Hyderabad, India.

Address for correspondence:

Dr. S. Vishnupriya, Department of Genetics, Osmania University, Hyderabad, India.
E-mail: sattivishnupriya@gmail.com

Abstract

Background: *TP53*, located on chromosome 17p13, is one of the most mutated genes affecting many types of human cancers. Thus, we aimed at investigating the association of SNPs in *TP53* gene with chronic myeloid leukemia (CML). **Materials and Methods:** A total of 236 CML and 157 control samples were analysed for mutations in *TP53* gene using polymerase chain reaction followed by direct sequencing. **Results:** Sequencing analysis for mutations in exons 7–9 of the *TP53* gene revealed four SNPs, three in intron 7 (C14181T, T14201G, and C14310T) and one SNP in intron 6 (A13463G) of *TP53* gene. The mutation C14181T is located at position 72 base pairs downstream of the 3'-end of exon 7 of the *P53* gene. This mutation is in complete linkage disequilibrium with a T14201G mutation, 20 base pairs further downstream occurring at position 14201. This mutation occurred only in the presence of C14181T mutation and these mutations showed association with advanced phase and cytogenetic poor response. Another two novel mutations, C14310T in intron 7 and A13463G in intron 6 were also found to be associated with cytogenetic poor response. **Conclusion:** Our study suggests that *TP53* intronic SNPs might have a strong influence on disease progression and poor response in CML patients.

Key words: Association, chronic myeloid leukemia, progression, response, single nucleotide polymorphism, *TP53* gene

INTRODUCTION

TP53 gene, located on chromosome 17p13, consists of 11 exons coding for an mRNA of 2.2–2.5 kb and approximately 53 kDa protein of 393 amino acids. *TP53* is the most important tumor suppressor gene that is involved in many pathways such as apoptosis, cellular transcriptional regulation, and cell cycle control.^[1,2] Mutations in the *p53* gene occur in approximately 50% of all human tumors.^[3] It is one of the most mutated genes observed in many types of human cancers.^[4,5] The majority of both sporadic and germline *TP53* mutations found in cancer cells were missense point mutations, occurring mainly in

the DNA binding domain of the protein.^[6] *P53* is a highly conserved gene and exons 5–8 contain four of the five evolutionary conserved domains of *p53* gene encoding amino acids 117–286. The majority of the reported mutations were found in this region.^[7]

The frequency of *p53* mutations in hematological malignancies were reported to be relatively low compared to other tumors, but the incidence was found to be increased in some cases with disease progression.^[8,9] It is well known fact that *p53* gene alterations might play a role in the progression of the chronic myeloid leukemia (CML), in fact close relation between myeloid blast crisis with loss of a 17p and *p53* gene point mutations was reported^[10] and 30% of the blast crisis patients were reported to carry *p53* gene mutations.^[11]

P53 polymorphisms were also reported in the intronic regions, which are thought to have regulatory roles in gene expression leading to susceptibility to cancer.^[12] Intronic variants might affect gene regulation through aberrant splicing or through disruption of critical DNA–

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protein interactions.^[13] Previous studies reported that polymorphism in intron 6 was significantly associated with increased risk for several cancers such as GI (gastrointestinal) tumors, breast, Li–Fraumeni syndrome, thyroid, and ovarian.^[14–17] These reports suggest that intronic mutations affect the stability of the *p53* protein and thereby its growth-suppression function. This study reports mutations in intron 6 and 7 of *TP53* gene in CML patients.

MATERIALS AND METHODS

This study comprises of 236 CML cases reported at Nizam's Institute of Medical Sciences, Hyderabad, during 2004–2006. A total of 157 age- and sex-matched healthy individuals without family history of cancer were selected to serve as a control group. Informed consent was taken from all the individuals recruited for the study, after obtaining ethical committee clearance. Five milliliter of blood samples were collected in EDTA vacutainers from both the CML patients and control groups. Patients clinical data like phase of the disease and cytogenetic response was noted from the tumor registry file with the help of oncologist. Response status was classified into major (1–35%), minor (35–65%), and poor (above 65%) based on the percentage of ph+ve cells and duration of response to imatinib therapy. Genomic DNA was isolated by using the salting-out method^[18] and used for mutational analysis.

A 964 bp fragment was amplified using a set of primers, forward: 5'-CTG CTT GCC ACA GGT CTC-3' and reverse: 5'-GAC AAT GGC TCC TGG TTG TA-3' covers both exonic and intronic regions from exons 7 to 9. These exons were located in highly conserved domains of DNA binding region of *TP53* gene, this region carries majority of mutations. Polymerase chain reaction (PCR) master mix (100 μ l for 10 samples) contains template DNA-50 ng, PCR buffer-10 μ l, MgCl₂-4 μ l, dNTPs-5 μ l, primer 2 μ l of each (5 picomoles), taq Polymerase-3 U, MilliQ water-65 μ l. PCR was carried out for 36 cycles with initial denaturation at 94 °C for 2 min followed by denaturation at 95 °C for 1 min, annealing at 58 °C for 45 s, extension at 72 °C for 2.30 min and final extension at 72 °C for 7 min. After PCR all the products were checked on 1.5% agarose gel for the presence of amplification. The amplified samples were subjected to direct sequencing (ABI Prism 3730 DNA Analyser).

Statistical analysis

The χ^2 -test was used to assess the significance of any difference in the prevalence of *TP53* polymorphisms between the CML patients and controls. Allele frequencies were calculated through the counting method. Odds ratio was estimated to calculate the relative risk for each

genotype to develop disease. Statistical significance was taken as $P < 0.05$.

RESULTS AND DISCUSSION

Intronic mutations are known to affect the stability of the *p53* protein and thereby its growth-suppression functions. Sequencing of 7–9 exons of *TP53* gene showed four SNPs, three in intron 7 and one in intron 6. The first C \rightarrow T SNP is located at position 14181, 72 base pairs downstream of the 3'-end of exon 7 of the *P53* gene. This SNP is in complete linkage disequilibrium with a T \rightarrow G mutation, 20 base pairs further downstream occurring at position 14201.^[19] The T14201G SNP occurred at the enhancer site of intron but did not abolish it. This mutation occurred only in the presence of C14181T mutation [Figures 1 and 2]. All the samples with C \rightarrow T mutation had T \rightarrow G mutation indicating that these mutations belong to the same allelotype and might have resulted from the same mutational event. Previous findings showed that there is complete linkage between C at 14181 and T at 14201 and between T at 14181 and G at 14201.^[20,21] This study on CML is in accordance with earlier reports.

The mutant homozygous haplotype TT-GG was observed in both CML cases (4.66%) and controls (8.92%) [Table 1]. In this study we could not find any significant association of CML with mutant TT-GG haplotype when compared to controls. Previous studies on acute myeloid leukemia and urinary bladder cancer also showed no significant association between mutant haplotype in intron 7 and cancer development.^[19] With respect to age at onset and sex of the proband, we could not find association with mutant haplotype. Odds ratio was estimated to calculate the relative risk for each haplotype to develop CML, odds ratio could not revealed any association between the intron 7 haplotypes and disease group [Table 2].

The frequency of mutant haplotype (TT-GG) was found to be elevated in patients with advanced phase blast crisis (9.5%) compared to chronic (4.2%) and accelerated (5.3%) phase of the CML. With respect to drug (Imatinib) response status, poor cytogenetic responders (10.5%) showed elevated frequency of TT-GG mutant haplotype as compared to major (3.3%) and minor (3.8%) responders [Table 1]. The significant association of intron 7 polymorphism (TT-GG mutant haplotype) with advanced phase and poor cytogenetic response suggests that these intronic mutations might have strong influence on disease progression and drug response.

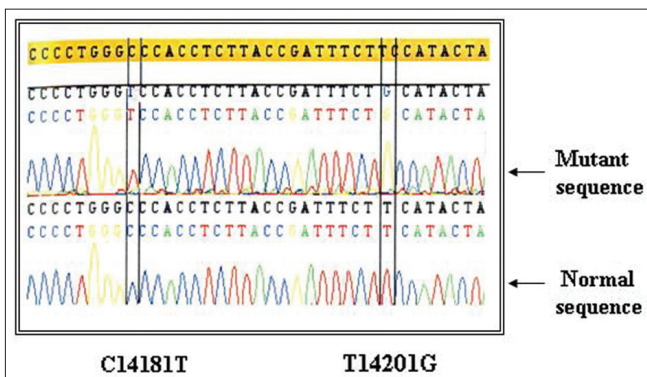
Two novel mutations were observed in our study, one in intron 6 (A13463G) and other in intron 7 (C14310T) one in

Table 1: Distribution of TP53 gene polymorphisms C14181T/T14201G with epidemiological and clinical variables

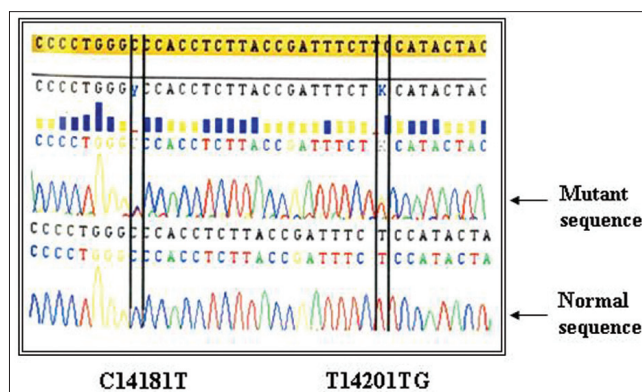
Parameters	CCTT No (%)	CTTG No (%)	TTGG No (%)	Allele frequency	
				C/T	T/G
Cases (236)	170 (72.0)	55 (23.31)	11 (4.66)	0.84	0.16
Controls (157)	104 (66.2)	39 (24.84)	14 (8.22)	0.79	0.21
$\chi^2 = 3.23$; df = 2, $P = 0.199$					
Age of the proband					
<20 years (23)	12 (52.2)	8 (34.8)	3 (13.0)	0.7	0.3
20–30 years (57)	45 (78.9)	11 (19.3)	1 (1.8)	0.89	0.11
30–40 years (54)	37 (68.5)	11 (20.4)	6 (11.1)	0.79	0.21
>40 years (102)	76 (74.5)	25 (24.5)	1 (1.0)	0.87	0.13
$\chi^2 = 16.058$; df = 6, $P = 0.013$					
Sex of the proband					
Males (161)	117 (72.7)	37 (23.0)	7 (4.3)	0.84	0.16
Females (75)	53 (70.7)	18 (24.0)	4 (5.3)	0.83	0.17
$\chi^2 = 0.158$; df = 2, $P = 0.925$					
Clinical phase of CML					
Chronic (189)	137 (72.5)	44 (23.3)	8 (4.2)	0.84	0.16
Accelerated (19)	13 (68.4)	5 (26.3)	1 (5.3)	0.82	0.18
Blast Crisis (21)	16 (76.2)	3 (14.3)	2 (9.5)	0.83	0.17
$\chi^2 = 1.986$; df = 4, $P = 0.738$					
Cytogenetic response					
Major (123)	87 (70.7)	32 (26.0)	4 (3.3)	0.84	0.16
Minor (26)	22 (84.6)	3 (11.5)	1 (3.8)	0.9	0.1
Poor (38)	27 (71.1)	7 (18.4)	4 (10.5)	0.8	0.2
$\chi^2 = 6.19$; df = 4, $P = 0.185$					

Table 2: Distribution of odds ratio

Odds ratio (95% CI)	Cases/controls	Sex of the proband
CCTT/CTTG	0.896 (0.535–1.390)	0.931 (0.486–1.783)
CCTT/TTGG	0.480 (0.210–1.098)	0.792 (0.222–2.824)
CTTG/TTGG	0.557 (0.228–1.356)	0.851 (0.220–3.288)

**Figure 1: Intronic haplotypes of TP53 gene C14181T and T14201G (Homozygous mutation)**

each of CML patients but none in controls [Figures 3 and 4]. Patients with A13463G mutation had minor cytogenetic response with age at onset of 42 years. Another CML

**Figure 2: Intronic haplotypes of TP53 gene C14181T and T14201G (Heterozygous mutation)**

patient having C14310T mutation had poor cytogenetic response and the age at onset was found to be 45 years. Both of these patients had normal haplotype in intron 7. Previous studies also reported TP53 intronic changes in 30% of the blast crisis samples and 12% in chronic phase.^[22]

CONCLUSIONS

TP53 mutations in intronic region may initiate aberrant

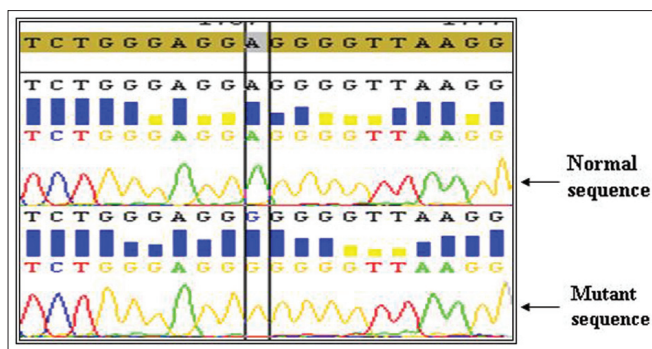


Figure 3: Homozygous mutation A13463T (intron 6)

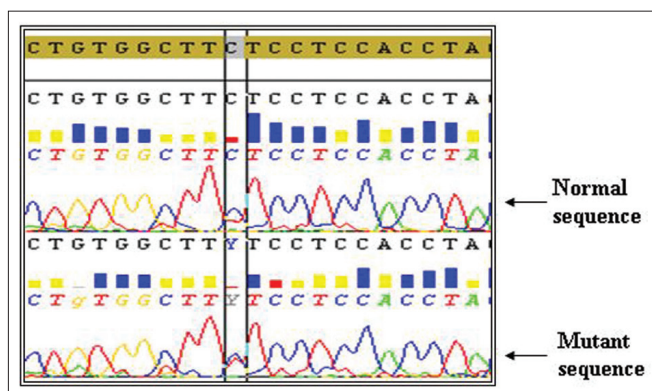


Figure 4: Heterozygous mutation C14310T (intron 7)

pre-messenger mRNA splicing, producing an mRNA that may be translated into defective *p53* protein which increases the likelihood of a deleterious phenotype that inhibits the apoptotic pathway and prolongs cell survival. Our study suggests that TP53 intronic SNPs might have strong influence on disease progression and poor response in CML patients. Hence, the study would be helpful in evaluating the progression and response status.

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